IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of TERRELL, Ross C.	Group Art Unit: 125
Serial No. 07/010,106	Examiner: J. Goldberg
Filed: February 2, 1987	Atty. Dkt.: PP4351
For: ANESTHETIC COMPOSITION AND METHOD OF USING THE SAME	Date: January 19, 1988

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

DECLARATION UNDER RULE 132

Sir:

I, Edmond I. Eger, II, MD, hereby declare and say as follows:

I am currently a Professor of Anesthesia and Vice Chairman for Research, with the University of California, San Francisco.

I received my B.S. Degree from the University of Illinois, an M.D. degree from Northwestern University, and specialty certification by the American Board of Anesthesiology.

I am a member of the Anesthesia Research Foundation (Secretary/ Treasurer), American Society for Pharmacology and Experimental Therapeutics, Association of University Anesthetists, International Anesthesia Research Society, Ralph M. Waters Award Commission Member, and Fellow of the Faculty, Royal College of Surgeons (England and Ireland).

I have authored or co-authored over 279 journal articles and four books, including Eger E.I. II, Anesthetic Uptake and Action, Baltimore, Williams and Wilkins (1974).

I have been a visiting Professor at numerous Universities since 1973, including the Johns Hopkins University, University of Pennsylvania, Mayo Clinic, Stanford University, Harvard University, Columbia-Presbyterian Hospital, and recently (1986) University of Umea, Sweden.

I am familiar with the invention disclosed and claimed in the above-identified patent application, S.N. 07/010,106, filed on February 2, 1987 for an Anesthetic Composition and Method of Using the Same.

I am familiar with the prosecution of said application in the United States Patent Office and the prior art cited in the course of the prosecution;

I have found, based on the studies described in detail below, that following administration of the claimed composition comprising 2-(difluoromethoxy)-1,1,1,2-tetrafluoroethane or $CHF_2OCHFCF_3$ (hereinafter referred to as "I-653"), awakening was more rapid than awakening from the currently available anesthetic isoflurane. In studies of these two agents in rats given 0.4, 0.8, 1.2 or 1.6 MAC for 2.0 hrs. or 1.6 MAC for 0.5 or 1.0 hrs. at a given dose and duration, awakening was more rapid with I-653 than with isoflurane. For example, recovery of muscle coordination at 1.2 MAC administered for two hours, required 4.7 ± 3.0 min (mean ± standard deviation) with I-653, compared to 23.2 ± 7.6 min. with isoflurane.

I declare that under my direction and to the best of my knowledge, the following experimentation was carried out and the following data and results obtained:

Experimental Materials and Methods:

This study was approved by the University of California, San Francisco Committee on Animal Welfare. Specific-pathogen-free, three-month-old, male Sprague-Dawley rats weighing 270-340 g (at the time of study) were purchased from Charles River Laboratories. Each was housed individually and had continuous access to standard rat chow and tap water before being studied.

Before being accepted for study, each rat passed a test of motor coordination (rotarod test) requiring that he maintain his position for five minutes on a six-cm (diameter) rod (Ugo Basile Rota-Rod Treadmill for Rats 7700). The test was imposed twice before anesthesia: first, one to three days after arrival at USCF, and second, within two hours before anesthesia. Each rat was given up to ten opportunities to pass the test at these trials. Rats failing the test (approximately five percent) were discarded.

Because we had a limited supply of I-653, we used a closed anesthetic system for all agents studied. The system was connected to a ventilator that alternately (20-30 times per minute) applied positive and negative pressure to a bag-in-box. The bag had a nominal volume of 500 ml. This arrangement provided the to and from movement of gases in the system needed to eliminate carbon dioxide and to assure adequate mixing of anesthetic newly introduced into the system. Gas from the bag-in-box moved past an injection port and an oxygen inflow port (the latter used only before the start of each study to flush the system with oxygen) and through the first of two CO_2 adsorbers. Anesthetics were injected through the injection port, as needed, to maintain the desired anesthetic concentrations. The port was sealed with a stopcock when not in use. The adsorbers were charged for each study with 50-55 g of fresh, commercial (Sodasorb*) soda lime containing 15 g water per 100 g soda lime. Blasts of air were used to clear dust from the soda lime prior to introducing both adsorbers into the closed system.

The gas issuing from the first adsorber was divided into four streams, each of which was directed to a chamber designed to contain a single rat. The ends of each chamber were sealed with rubber stoppers that had two openings or "pass

throughs", one in the front for sampling gases (near the rat's head), and one at the rear for the rectal temperature probe. The sampling ports were sealed with stopcocks when not in use. All chambers were 27 cm long and has an internal diameter of 6.25 cm, which provided sufficient space for the rat to crawl into the chamber, but did not allow him to turn around.

The gases issuing from the rear of each of the four chambers were combined and directed through the second CO_2 adsorber, then traveled through a tube to a reservoir bag. The tube had a side port that permitted the addition of oxygen through a one-way valve from an oxygen delivery catheter. The oxygen delivery catheter was connected to the stem of a T-tube. One arm of the T was connected to an oxygen source and the other arm to a reservoir tube. During each study, the oxygen bypass flow through the reservoir arm of the T was 300 ml per min. Excess oxygen flow escaped through an underwater seal (1-3 cm water pressure).

To determine anesthetic concentrations, we used a Gow Mac Model 750 gas chromatograph equipped with a 30 meter long, fused silica open tubular capillary column (0.53-mm internal diameter) coated with a five micron thick layer of methylsilicone oil (J&W Scientific DB-1). A nitrogen carrier stream of six ml/min was directed through the column with a "make-up" flow of nitrogen of 40 ml/min delivered to the detector. A flame ionization detector at 200°C was supplied by hydrogen at 40 ml/min and by air at 280 ml/min. Samples were injected with a 0.05 ml gas sample loop. The chromatograph was calibrated with secondary tank standards that had been calibrated with primary standards produced by injection of a liquid aliquot of anesthetic into a flask of known volume. Because the concentrations of I-653 required for anesthesia exceeded the linear range of our gas chromatograph, we reduced the

concentration into the linear range by diluting each sample by at least a factor of 10.

Each rat was put in his chamber, after which the rectal probe was placed and secured with paper tape. A flow of 5-L/min or greater of oxygen was then directed through the chambers for 10 min or longer, the reservoir bag being removed during this period of flushing, to ensure denitrogenation. The system was them sealed except for the opening to the oxygen bypass.

Anesthetic was introduced in an amount calculated to produce a rapid rise to the desired concentration. The concentrations actually achieved were determined from gas samples obtained from the chambers in sequence, usually at 10-15 min intervals.

During anesthesia, the rectal temperature of each rat was maintained between 37.5°C and 38.5°C by applying heat (infrared lamps) or cold (ice) to the outside of the chambers. Carbon dioxide concentrations were measured during each study (infrared analysis with a Beckman LB-2 analyzer) and usually were between 0.7% and 1.0%, the highest measuring 1.5%. Oxygen concentrations, also measured during each study (Beckman E2 Pauling meter, calibrated with nitrogen and oxygen each day), were never less than 75%.

At the end of the specified period of each anesthetic study, the reservoir bag was removed and oxygen flushed through the system for a few seconds before the rats were removed from their chambers and placed supine. Temperature probes were left in place and heat was applied to the rats to help maintain their temperature; temperatures invariably decreased slightly after removal from the tubes. The time each rat required to righting himself twice, righting defined as turning to position all four feet on the table, was measured and recorded.

Immediately after the second righting, the temperature probe was removed, and each rat was tested for the ability to remain upright on the rotarod for 60 secs. The test was repeated thereafter at intervals determined by multiples of three (i.e., at 3, 6, 9, 12 min, etc.) from the time anesthesia was discontinued. Each rat was given two chances to pass the rotarod test at each interval. When he had maintained his position atop the rotarod for 60 consecutive secs, the study was concluded.

No rat was given more than one concentration and one anesthetic. The concentrations and durations for isoflurane were 0.4 MAC, 0.8 MAC, 1.2 MAC, and 1.6 MAC for two hrs, and 1.6 MAC for 0.5 hrs and 1.0 hrs (Table 1). The 0.4 MAC determination was not made with I-653 because the rats awakened immediately at the 0.8 MAC level. The MAC values assumed were 5.7% I-653 and 1.4% isoflurane.

Data Analysis and Results:

The results for times to recovery were subjected to a determination of mean and standard deviation values. A regression analysis was applied separately to the data obtained at constant duration of anesthesia and at constant anesthetic dose.

The desired anesthetic concentrations were rapidly achieved in all cases. The average concentration obtained for I-653 for the groups of four rats was 96.9 $\pm$ 4.4% (mean $\pm$ SD; N = 11) of the desired concentration, and 101.2 $\pm$ 8.4% for isoflurane (N = 13). The anesthetic effect was apparent soon after injection of agent into the system and was associated with a tendency toward a decrease in temperature, prevented by heating the chambers with infrared lamps.

At a given MAC multiple and duration, recovery from I-653 was more rapid than isoflurane (Tables 2 and 3; attached FIGS. 1-3). Results for the the recovery of the righting reflex were more variable than those for recovery of motor coordination (the rotarod test) (Tables 2 and 3).

It appears that at a concentration of 0.8 MAC or greater, the product of anesthetic duration and anesthetic dose produced the same time to recovery regardless of whether the concentration produces a light or deep level of anesthesia. That is, at anesthetizing concentrations, the MAC hours of anesthesia determine the time of recovery.

Recovery was most rapid after anesthesia with I-653 regardless of the duration of anesthesia and the inspired anesthetic concentration. This outcome indicates a particular advantage to the use of I-653 in anesthesia for outpatient surgery where shift recovery of coordination should enhance the safety of ambulating patients. A more rapid recovery should decrease the danger of patient injury from falling and reduce the time to the safe operation of equipment requiring manual dexterity such as driving a car. Our results also suggest that the administration of deep levels of I-653 to produce hypotension or muscle relaxation should not unduly prolong recovery from anesthesia.

Table 1. Anesthetic Concentrations and Durations Tested (+)

Duration	Anesthetic Concentration (MAC)			
	0.4	0.8	1.2	1.6
0.5 hrs	-	-	-	+
1.0 hr	-	-	-	+
2.0 hr	+	+	+	+

Table 2. Minutes to Righting

Hrs	MAC	I-653	Isoflurane
0.5	1.6	2.4 ± 1.6	7.5 ± 5.7
1.0	1.6	3.5 ± 2.5	8.2 ± 5.1
2.0	1.6	4.4 ± 2.3	9.7 ± 4.5
2.0	1.2	1.1 ± 0.7	10.1 ± 8.0
2.0	0.8	0.3 ± 0.8	8.8 ± 9.0
2.0	0.4	---	0.9 ± 2.2

Values are expressed as mean ± SD.

N = 8 for all groups except those given 1.6 MAC for 2.0 hrs. For this last group, N = 12.

Table 3. Minutes to Pass Rotarod Test

Hrs	MAC	I-653	Isoflurane
0.5	1.6	3.4 ± 1.2	11.6 ± 4.7
1.0	1.6	4.8 ± 2.1	14.6 ± 1.9
2.0	1.6	9.8 ± 4.1	35.8 ± 7.6
2.0	1.2	4.7 ± 3.0	23.2 ± 4.7
2.0	0.8	1.4 ± 1.5	14.2 ± 7.6
2.0	0.4	---	3.4 ± 1.2

Values are expressed as mean ± SD.

N = 8 for all groups except those given 1.6 MAC for 2.0 hrs. For this last group, N = 12.

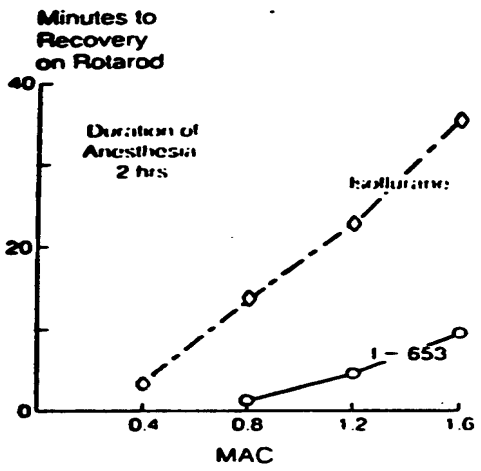

FIG. 1

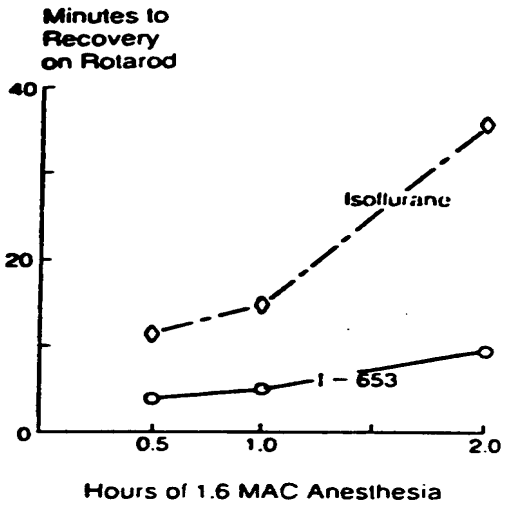

FIG. 2

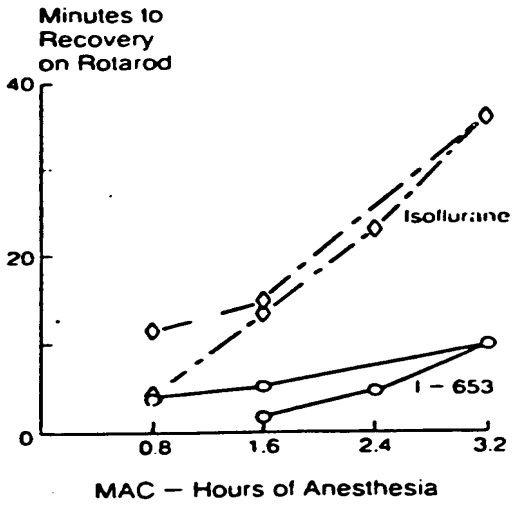

FIG. 3

I declare further that all statements made in this declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

21 Jan 88
Date

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